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Quantitative Branching Geometry of the Vascular System of the Blue Crab, *Callinectes sapidus* (Arthropoda, Crustacea): A Test of Murray's Law in an Open Circulatory System

DAVID MARCINEK[1] AND MICHAEL LABARBERA[2]

[1]*Biology Department, Kalamazoo College, Kalamazoo, Michigan 49006, and* [2]*Department of Organismal Biology and Anatomy, The University of Chicago, 1025 East 57th St., Chicago, Illinois 60637*

Abstract. Murray's law predicts that there will be a radius-cubed relationship between the parent and daughter vessels of a branching system of vessels that carry the flow of a fluid, a relationship that theoretically minimizes the costs of building, maintaining, and operating the system. The vascular system of the blue crab, *Callinectes sapidus*, was replicated by corrosion casting at physiological pressures; vessel diameters were measured off the casts and used to calculate a junction exponent for each branch point. This study is the first quantitative description of the vascular branching geometry in an open circulatory system. The mean value derived from the arctan-transformed junction exponent distribution, 3.020, was not significantly different from the value of 3 predicted by Murray's law. The phylogenetic distance of arthropods from the animals previously studied in this context, sponges and mammals, is evidence for three independent evolutions of this branching relationship in biological fluid transport systems.

Introduction

Organisms and their component cells depend on exchange with their surroundings for nutrient procurement, excretion, respiration, and temperature regulation. Animals commonly meet the demands of exchange with a system of branching pipes, within which there is convective flow of a fluid. Two major types of fluid transport systems are seen in animals (LaBarbera, 1990). In one, used by many suspension feeders, fluid is pumped from an external source past the animal's filtering structures. The second, an internal transport system, is familiar as the circulatory system of most "higher" animals. Circulatory systems can be further divided (LaBarbera and Vogel, 1982) into those in which the circulating fluid is completely contained in a network of vessels (closed circulatory systems) and those in which the circulating fluid leaves well-defined vessels and flows through sinuses to bathe the tissues (open circulatory systems). For a fluid transport system to function effectively, there must be continuous flow of the fluid, which results in large volumes of fluid being pumped at substantial cost to the animals (LaBarbera, 1994).

Is there a design principle for transport of a fluid that both minimizes the cost of volumetric flow and is general enough to apply across the diversity of fluid transport systems observed in biology? This question was first treated over a century ago by Thomas Young in his 1808 Croonian lecture (Sherman, 1981). Since then, several physiologists have addressed the question of an ideal branching pattern, but the "clearest and most general approach" (Sherman, 1981) is that of Murray (1926).

The derivation of "Murray's law" [see Sherman (1981) and LaBarbera (1993) for details] assumes that there is laminar flow of a fluid through circular pipes, an assumption that is not grossly violated in biological fluid transport systems. If the velocity profile is fully developed ("Poiseuille flow," a stable parabolic velocity profile; see

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[1] Present address: Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

[2] To whom all correspondence should be addressed.

Vogel, 1981), and the fluid is Newtonian (*i.e.*, the viscosity of the fluid is independent of the characteristics of the flow), then the Hagen-Poiseuille equation (Vogel, 1981) states that, for a given volumetric flow rate, the resistance to flow is inversely proportional to the radius of the vessel to the fourth power. This relationship would imply that the cost of moving the fluid can be minimized by maximizing the radius of the vessels. However, Murray's derivation also assumes that there is some cost proportional to the volume of the system; this cost might be the cost to build and maintain the vessel walls (Sherman, 1981), the formed elements in the blood (Murray, 1926), or any other volume-related cost (LaBarbera and Boyajian, 1991). Minimizing these volume-related costs by decreasing vessel radius necessarily increases the costs associated with moving the fluid. The total cost can be minimized only if the volumetric flow rate through a vessel in the system is maintained proportional to the cube of the vessel radius (Murray, 1926), a relationship known as Murray's law. This form of Murray's law is supported by a number of empirical studies on the relationship between volumetric flow rate and vessel radius in the vascular systems of birds and mammals [see LaBarbera (1994) for a review].

Murray's functional relationship makes a clear morphological prediction. At a branch point in a fluid transport system, where a single parent vessel gives rise to two or more daughter vessels, the volumetric flow rate in the parent vessel must be equal to the sum of the volumetric flows in the daughter vessels (the "principle of continuity"; Vogel, 1981). In the equation

$$r_0^x = r_1^x + r_2^x + \cdots \cdot r_n^x$$

where r_0 is the radius of the parent vessel and $r_1 \cdot \cdots \cdot r_n$ are the radii of the daughter vessels, Murray's law thus predicts that x, the junction exponent, will have a value of 3. (Note that other values for the junction exponent are possible. A system in which flow velocity was maintained constant in all vessels would have a junction exponent of 2; a system in which resistance to flow was maintained constant would have a junction exponent of 4.) A radius-cubed relationship of daughter to parent vessels is supported by empirical evidence for multiple species of mammals (Sherman, 1981; LaBarbera, 1990, 1994) and sponges, including Devonian stromatoporoids (LaBarbera, 1990, 1993; LaBarbera and Boyajiian, 1991), an extinct group of sponges. Given the extreme phylogenetic distance between sponges and mammals, the agreement with Murray's law observed in these animals is probably not due to chance occurrence or a single evolution of this system (LaBarbera, 1990).

While previous work provides data on two functionally highly divergent systems (the trophic fluid transport system of sponges and the closed circulatory system of vertebrates), to date there are no available data on the applicability of Murray's law to open circulatory systems. The present study on the vasculature of the decapod crustacean, *Callinectes sapidus*, represents the first quantitative analysis of vessel branching in an open circulatory system.

Materials and Methods

Maryland blue crabs, *Callinectes sapidus* Rathbun (98–185 g wet weight), were purchased from a local fish market on the same day they were received. Because the crabs were shipped on ice, they were placed in plastic containers containing aerated 4–5°C artificial seawater (salinity 32–34‰) and allowed to warm gradually (2 h minimum) to room temperature to minimize temperature shock. After thermal acclimation, the crabs were transferred to 300-l aquaria at room temperature. Because of their aggressive nature, the blue crabs were generally isolated in plastic mesh cages submerged in the aquaria; when isolation was not possible, their chelipeds were bound.

The mortality rate during acclimation was 20–40%, and it reached 50% after two days; animals still alive after two days generally survived until used (1–14 days). The heart and major blood vessels were located, during dissection, with the help of McMahon and Burnett's (1990) description of the brachyuran circulatory system.

Surviving animals were anesthetized by injection of 0.2 ml of 3–5% procaine in distilled water into the coxal joint of a rear periopod or swimming leg; procaine is reported to minimally influence cardiac parameters in crabs (Oswald, 1977). After being injected, the animal exhibited a short period (20–30 s) of excitation followed by retraction of its appendages and subsequent insensitivity to stimuli. We manually drilled a hole through the dorsal carapace, about one-third of the distance from its posterior edge, to access the heart. A 20-gauge hypodermic needle attached by 20 cm of PE-90 catheter tubing to a Gould P32 pressure transducer was inserted into the heart; cardiac pressures were recorded on a Gould 2200 strip chart recorder. Normal heartbeat patterns and systolic pressures were successfully recorded from three anesthetized blue crabs; a typical pressure recording is shown in Figure 1. Measured systolic pressures were 11–14 cm H_2O (mean

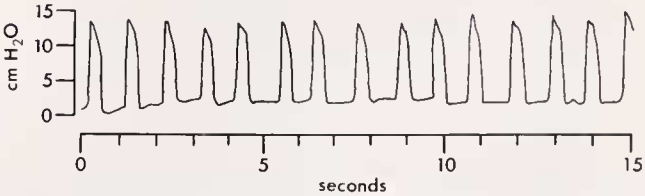

Figure 1. A typical pressure recording from the heart of a blue crab. The vertical scale is pressure (in cm H_2O; 1 cm H_2O = 0.098 kPa); the horizontal scale is time (in seconds).

= 13.1 ± 0.3 cm H_2O), and these pressures (=1.08–1.37 kPa) were maintained throughout circulatory system perfusion and vascular filling. Measured systolic pressures were virtually identical to those reported for other crabs of comparable size (*Cancer productus*—14.1 cm H_2O, *C. maenas*—14.0 cm H_2O; McMahon and Burnett, 1990). During cardiac pressure measurements and throughout the vascular casting procedure, the crab was submerged in seawater.

After pressure measurements were completed, the 20-gauge needle was removed and replaced with an 18-gauge needle connected, via PE-160 tubing, to a 50-ml syringe body (the perfusion reservoir). Hemolymph was rinsed from the vascular system using cold (4–5°C) artificial seawater to inhibit clotting (J. L. Wilkins, Univ. of Calgary, pers. comm.); the air-water interface in the perfusion reservoir was maintained 10–14 cm above the level of the water covering the crab to ensure a proper pressure gradient. One of the posterior periopods was excised at the coxal joint to allow the hemolymph to drain. In some cases, uranine dye was added to the rinse solution to help identify when the rinse emerged from the animal. Rinsing times and volumes ranged from 16 to 36 min and 32 to 53 ml, respectively. When exsanguination appeared to be complete, the vascular system was perfused for 20–30 min with cold (4–5°C) 10% buffered formalin in seawater; the fluid level in the perfusion reservoir was maintained to ensure fixation at physiological pressures.

During exsanguination, the crabs occasionally displayed spontaneous, random movement of their appendages. We attempted to maintain complete anesthesia in some specimens by adding 2–3 ml of procaine solution to the rinse solution; spontaneous appendage movement was reduced, but the vascular casts from these specimens were less complete or without well-defined vessels. The results reported here, therefore, were obtained exclusively from specimens without supplementary anesthesia.

Bateson's #17 (Polysciences, Inc.), diluted with methylmethacrylate to reduce the viscosity of the casting medium (Levesque *et al.*, 1979; Lametschwandtner *et al.*, 1990), was used to fill the vascular system for corrosion casting. Components were mixed according to the manufacturer's protocol in the following proportions: 50 parts Bateson's monomer, 10 parts catalyst B, 1 part promoter C, 50 parts methylmethacrylate (Levesque *et al.*, 1979). Immediately after being mixed, casting compound was poured into a 10-ml syringe attached to an 18-gauge needle via a short segment of catheter tubing; the tubing and needle were bled of air before being inserted into the heart. Because the density of the casting compound (1.079 g/ml) was about that of water, the meniscus of the uncured Bateson's was maintained 10–14 cm above the water covering the crab, ensuring normal systolic pressures in the heart. The casting compound continued to flow into the crabs' hearts for 25–32 min before polymerization stopped the flow; the volumes infused ranged from 5.2 to 9.4 ml.

Shrinkage of the modified Bateson's upon polymerization was measured by filling microcapillary tubes (1.4, 1.0, 0.6, and 0.4 mm internal diameter) with casting compound. After polymerization, the tube was broken and diameters for both the unbroken sections of the tube and casts were measured. The mean diameter shrinkage was 3.8% ± 0.5% (mean ± SE); there was no significant difference in shrinkage between tubes of different sizes (ANOVA: $F_{3,32} = 0.263$, $P = 0.85$).

The specimen was left in seawater overnight to allow the cast to fully polymerize. The exoskeleton was decalcified by immersing the specimen for 24 h in 20–25% acetic acid:H_2O solution; after sections of the decalcified cuticle were cut away and the body thoroughly rinsed of acid, the specimen was transferred to 30% KOH for soft tissue digestion (Lametschwandtner *et al.*, 1990). Maceration continued for up to three days; specimens were rinsed and the KOH solution was changed daily. After treatment in KOH, the remaining chitinous matrix was dissected away and the cast washed; casts were stored in distilled water.

The vascular casts were photographed on Kodak Technical Pan film using a Nikon FM 35-mm camera and MicroNikkor 55-mm macro lens. The cast was fixed to a universal stage and the stage adjusted to ensure that the parent and daughter vessels of the junction being photographed were coplanar and oriented perpendicular to the optic axis of the lens. A stage micrometer was photographed for scale. The negatives were projected at a total magnification of 330× and traced; linear dimensions were measured with a Summa Sketch II digitizing pad connected to an Apple Macintosh IIci. Because vessel diameter varies slightly along the length of the vessels, several diameters were measured along the length of each vessel segment; mean values were used to calculate junction exponents and predicted parent vessel diameters. Where possible, the total vessel segment length between junctions was also measured.

In many instances a junction had vessels projecting or curving into more than one plane; these junctions were excluded from the sample. A number of vessels in the casts appeared flattened, which we interpreted as indicating incomplete filling by the casting compound; these vessels were also excluded from the analysis.

Best fit values for junction exponents were determined to four decimal places with an iterative search program. Where the diameter of one of the daughter vessels was equal to or larger than that of the parent vessel, the junction exponent was considered undefined. Statistical tests of junction exponents, by definition, exclude consideration of junctions where the junction exponent is undefined. To test the full data set for consistency with Mur-

Figure 2. Corrosion casts of the blue crab vasculature. (A) The anterior dorsal region of the thorax and a cheliped before dissection of the cast from the exoskeleton. (B) A vasculature cast removed from the exoskeleton. The heart is the large mass at the bottom center of the photograph; the sternal artery, the major vessel in the midline of the cast, supplies the mandibular region and periopods in blue crabs (see Fig. 3). The arteries serving the tips of one of the chelae can be seen in the upper left.

ray's law, we also compared measured values for parent vessel diameters with parent vessel diameters predicted from the measured daughter vessel diameters, assuming a junction exponent of 3 (LaBarbera and Boyajian, 1991; LaBarbera, 1994). This test allowed inclusion of branch points where the junction exponent was undefined.

All numerical calculations and most statistical tests were performed with StatView II (ver. 1.04; Abacus Concepts, Berkeley, California). One sample and paired sign tests were run on StatView 4 (ver. 4.01; Abacus Concepts); confidence intervals for medians of untransformed junction exponent distributions were determined by bootstrap resampling with replacement (1000 runs) of the distributions using the program Resampling Stats (ver. 1.00; Resampling Stats, Arlington, Virginia). All analyses were run on a Macintosh IIci.

Results

Examples of blue crab vascular casts are given in Figure 2; a schematic diagram of a typical vascular tree is given in Figure 3. Vessel diameters were measured from 106 junctions, pooled from vascular casts of three crabs; 98 of these junctions exhibited dichotomous branching, and 8 possessed three daughter vessels. Vessel diameters ranged from 52 to 785 μm, with parent vessel diameters spanning nearly this full range (71–785 μm; Fig. 4). Segment lengths (the distance between branch points) could be accurately determined on only 159 segments (Fig. 5). Segment length/diameter ratios were extremely variable, ranging from 0.50 to 17.8; the mean value was 3.98 ± 0.62 (median = 3.21).

The frequency distribution of junction exponents was sharply peaked and right skewed (Fig. 6). The mode of this distribution appeared to be between 2 and 3, although both the mean and median gave higher values (Table I). Because the distribution was obviously skewed and significantly different from a normal distribution (Lillifors test; Table II), we used an arctan transformation (LaBarbera, 1994; Suwa *et al.*, 1963) to normalize the data (Fig. 7). The arctan-transformed frequency distribution was not significantly different from a normal distribution (Table II). Neither the median of the untransformed dis-

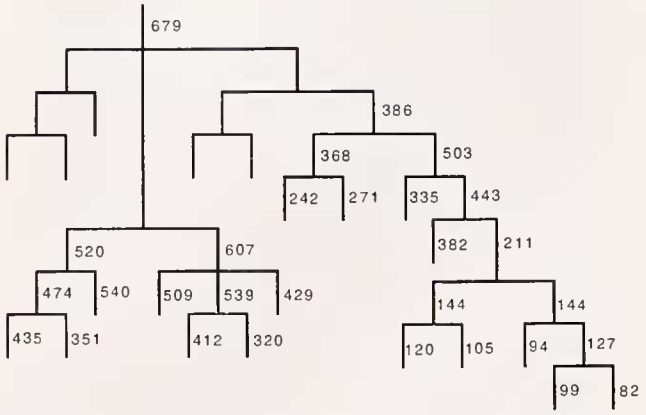

Figure 3. Branching topology and diameters (in μm) of the vessels supplying the mandibular region from a segment of the sternal artery (the parent vessel in this network) in blue crabs. Branches that lack diameter values could not be reliably measured. Note that dichotomous branching is most common, but junctions with more than two daughter vessels do occur.

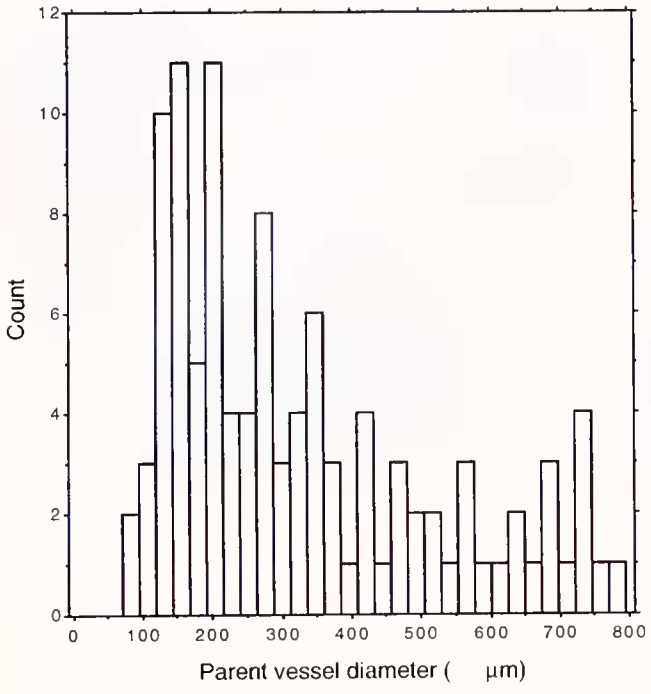

Figure 4. Frequency distribution of the parent vessel diameters (in μm) of the 106 junctions measured from vascular casts of three blue crabs.

tribution nor the (backtransformed) mean of the transformed distribution was significantly different from a value of 3 (Table I); based on confidence intervals, the mean of the untransformed distribution was significantly different from 3.

The pooled exponents were divided into quasi-arbitrary groups on the basis of anatomical location and diameter of the parent vessel (Table I); the particular groupings in Table I were chosen to maintain sample sizes approximately constant. Three groups were established on the basis of anatomical location of the junction—vessels of the anterior-ventral region, the vessels supplying the ventral regions of the thorax and the periopods, and the dorsolateral arteries (which supply the digestive gland and the peripheral thoracic arteries). All three of the groups showed frequency distributions of junction exponents that were significantly non-normal; the arctan-transformed junction exponent distributions were not significantly different from normal distributions (Table II). ANOVA of the transformed distributions ($F_{2,83} = 0.312$; $P = 0.73$) and Kruskal-Wallis rank tests of the untransformed distributions ($P = 0.64$) showed no significant differences between anatomical groups. Neither the medians of the untransformed distributions nor the backtransformed means of the transformed distributions were significantly different from a value of 3 (Table I); based on confidence intervals, one of the three means of the untransformed distributions was significantly different from 3.

Grouping the junction exponents on the basis of diameter of the parent vessel produced a similar pattern. Two of the four groups showed frequency distributions of junction exponents that were significantly non-normal, as was one of the size groups of arctan-transformed junction exponents (Table II); thus, ANOVA could not be used to test for differences between groups. However, Kruskal-Wallis rank tests of the untransformed ($P = 0.20$) and transformed distributions ($P = 0.27$) showed no significant differences between size groups. Neither the medians of the untransformed distributions nor the backtransformed means of the transformed distributions were significantly different from a value of 3 (Table I); among the means of the untransformed distributions, only the mean for the largest size group (431–785 μm) was significantly different from 3.

To include all measured junctions in the analysis (not just junctions where the exponent was defined), we used the measured diameters of the daughter vessels at each junction to predict a diameter for the parent vessel (Table III; a junction exponent of 3 was assumed in these calculations). Again, the data were grouped by anatomical location of the junction and parent vessel diameter. In every case, the mean ratio of the predicted diameter to the observed diameter was greater than 1, implying a tendency for the parent vessel to be slightly smaller than would be predicted by a junction exponent of 3 (or, equivalently, for the daughter vessels to be slightly larger

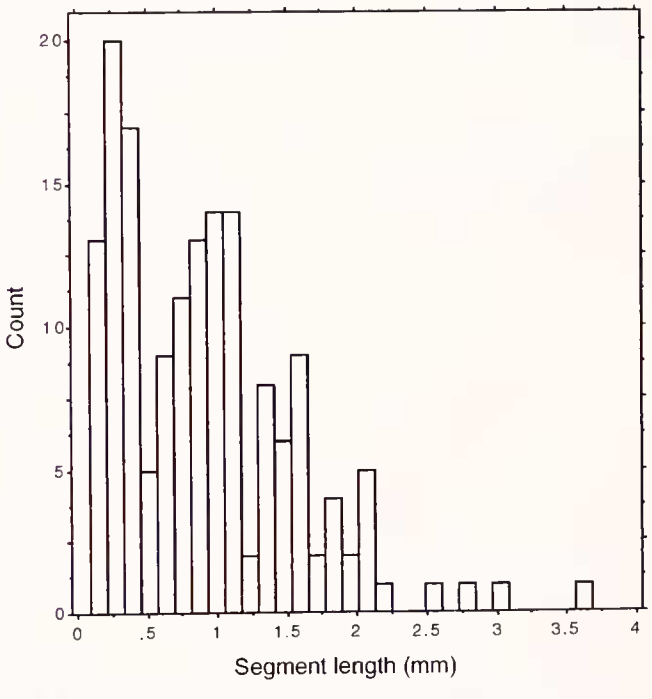

Figure 5. Frequency distribution of lengths of 159 vessel segments in the blue crab arterial system.

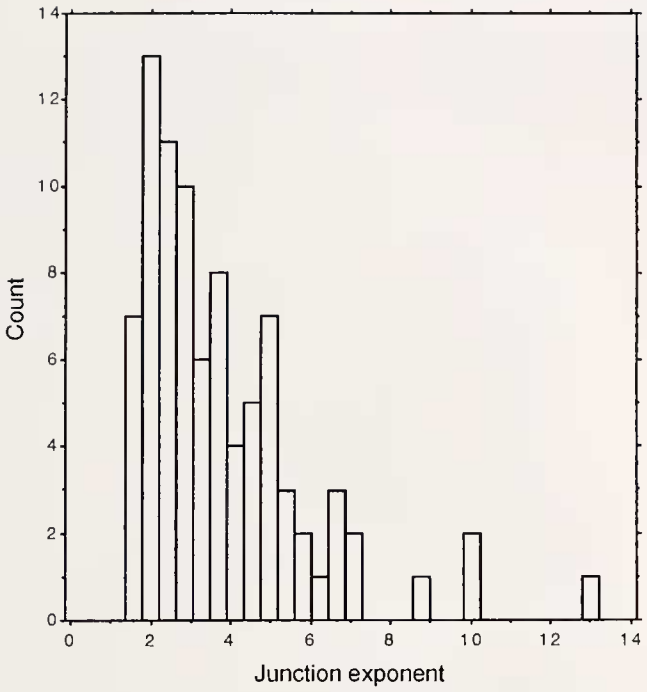

Figure 6. Frequency distribution of the junction exponents calculated from measured parent and daughter vessel diameters at 86 branch points in the arterial systems of three blue crabs. Note that the distribution is strongly right-skewed and non-normal (see Table II) but exhibits a mode at an exponent between 2 and 3.

than would be predicted by a radius-cubed relationship with the parent vessel). Although all ratios were consistently greater than 1, nonparametric two sample sign tests revealed significant differences between observed and predicted diameters only for three groupings: the full data set, the dorso-lateral vessels, and the largest parent vessels (Table III).

Discussion

Tests of Murray's law

The objective of this study was to provide a quantitative description of vessel branching in an open circulatory system and test the results against the idealized geometry of an "optimally designed" fluid transport system as predicted by Murray's law. Given the highly skewed frequency distribution of junction exponents (a feature also seen in the fluid transport systems of sponges and mammals; see LaBarbera, 1994), a simple mean is clearly a poor measure of central tendency of the junction exponent distribution. The large discrepancy between the mean and median of the distribution is a clear indicator of its non-normality, a conclusion confirmed by statistical analysis (Table II). We have provided two alternative measures, the median and the mean of arctan-transformed junction exponents, both of which are more robust indicators of central tendency than a simple mean. Both measures clearly indicate that the junction exponents of vessels in the arterial system of blue crabs are not significantly different from the value of 3 predicted by Murray's law. This conclusion is robust to divisions of the data by either anatomical location or vessel size (Table I). Although both mammals and fossil stromatoporoid sponges have been previously shown to exhibit a fluid transport system in quantitative agreement with Murray's law (data summarized in LaBarbera, 1994), this is the first demonstration that an animal with an open circulatory system exhibits such a vascular architecture.

Most previous quantitative tests of the anatomical predictions of Murray's law (*e.g.*, Suwa *et al.*, 1963; Hutchins *et al.*, 1976; Arts *et al.*, 1979; Wieringa *et al.*, 1988; Griffith and Edwards, 1990) have focused on branch points where junction exponents were (by our definition) defined. The discordance between Table I, where no statistically significant differences were found between the blue crab vasculature and Murray's law, and Table III, where significant differences were apparent, can clearly be attributed to the inclusion of junctions where the junction exponent was undefined, effectively changing the data set being tested. Although inclusion of all junctions yields statistically significant differences between the idealized and real system, the actual error is small; Murray's law predicts parent vessel diameters averaging only 5% larger than the measured vessels.

Proximate mechanisms for generating Murray's law systems

Our conclusion that the blue crab circulatory system approaches Murray's model is significant, not only because it is the first test of Murray's law in an open circulatory system, but also because of the evolutionary position of arthropods in relation to the groups previously examined in this context—sponges (including 375-million-year-old stromatoporoids) and vertebrates (LaBarbera, 1990, 1994; LaBarbera and Boyajian, 1991). The Arthropoda are a distinct evolutionary lineage, separate from both sponges and vertebrates, which are in turn distinct from one another (Turbeville *et al.*, 1991, 1992; Ballard *et al.*, 1992; Wainwright *et al.*, 1993), all three groups diverged before the development of their fluid transport systems (LaBarbera, 1994). Thus, approximations of Murray's law systems must have evolved independently at least three times and were in place at least 375 million years ago (LaBarbera and Boyajian, 1991). The convergent evolution of Murray's law fluid transport systems is probably driven by the substantial costs incurred in moving the large relative volumes these systems transport (see LaBarbera, 1994). Because even small changes in effi-

Table I

Measures of central tendency in the distributions of junction exponents in the blue crab arterial system

		Untransformed				Arctan transformed	
	n	Mean	*P*	Median	*P*	Mean	*P*
All vessels	86 (20)	3.758* (3.300–4.215)	—	3.129* (2.779–3.700)	0.75	3.020 (2.758–3.341)	0.89
Anatomical regions							
Anterio-ventral	29 (6)	4.014* (3.166–4.861)	—	3.079* (2.770–4.581)	>0.99	3.235 (2.767–3.887)	0.35
Ventral thorax, periopods	33 (4)	3.551* (2.901–4.202)	—	3.257* (2.230–3.888)	0.73	2.884 (2.484–3.429)	0.64
Anterio-lateral	24 (10)	3.732* (2.700–4.764)	—	2.999* (2.287–3.847)	>0.99	2.970 (2.500–3.630)	0.92
Parent vessel diameter							
<167 μm	23 (3)	3.785* (2.849–4.721)	—	3.113* (2.358–3.765)	>0.99	3.000 (2.469–3.762)	0.99
168–268 μm	22 (4)	3.529 (2.754–4.304)	0.17	3.036 (2.326–3.888)	>0.99	3.020 (2.587–3.602)	0.95
269–430 μm	19 (7)	3.518* (2.200–4.385)	—	2.144* (2.028–4.550)	0.36	2.634* (2.153–3.329)	—
431–785 μm	22 (6)	4.165 (3.302–5.029)	0.01	3.890 (3.033–4.923)	0.13	3.429 (2.829–4.294)	0.17

Data are presented for both the full data set and arbitrary subdivisions of the vessels according to anatomical location or parent vessel size. Three independent measures of central tendency are given: the mean and median of the original distributions and the mean of the distribution of the arctan transformed values (backtransformed to arithmetic units). In each cell of the table, the 95% confidence interval of the relevant metric is given in parentheses. Distributions that were significantly different from a normal distribution are indicated with an asterisk (see Table II). The numbers in parentheses in the second column represent the number of undefined junctions (see text) for each group. 95% confidence intervals for the medians were calculated by bootstrap resampling of the original data (see text). Where the distributions were not significantly different from a normal distribution, the means were tested against a hypothesized value of 3 using a one-sample *t*-test; medians were tested with a (nonparametric) one-sample sign test.

Table II

Lilliefors (Kolmogorov-Smirnov) tests of normality of the distributions of junction exponents in blue crabs

	n	Untransformed	Arctan transformed
All vessels	86 (20)	<0.0001	0.062
Anatomical regions			
Anterio-ventral	29 (6)	0.017	0.701
Ventral thorax, periopods	33 (4)	0.049	0.129
Anterio-lateral	24 (10)	0.012	>0.999
Parent vessel diameter			
<167 μm	23 (3)	0.018	0.908
168–268 μm	22 (4)	0.084	0.445
269–430 μm	19 (7)	0.001	0.005
431–785 μm	22 (6)	0.427	0.334

The values given are the probabilities that each distribution tested (see Table I) is statistically different from a normal distribution in shape. All untransformed distributions except two of the size categories (168–268 and 431–785 μm) are significantly different from a normal distribution; for the arctan transformed data, only the distribution of exponents from junctions whose parent vessels were between 269–430 μm in diameter was significantly different from a normal distribution.

ciency in these systems are amortized over the lifetime of the animal, selective pressures to decrease these costs are probably large.

LaBarbera (1990) suggested that convergent evolution of Murray's law systems has occurred because of the relative ease with which natural selection can exploit a local, cellular-level signal—hydrodynamic shear stress on the walls of the vessels—to generate an organism-spanning, near-optimal system. In a fluid transport system that follows Murray's radius-cubed relationship, the velocity profiles are geometrically similar everywhere in the system (Sherman, 1981); because velocity gradients at the wall of the vessel are identical, the hydrodynamic shear stresses on the walls of the vessels are identical everywhere in the system if fluid viscosity is constant. A number of workers (Zamir, 1977; Kamiya and Togawa, 1980; Kamiya *et al.*, 1988; LaBarbera, 1990) have suggested that, in the mammalian vascular system, the endothelial cells act as mechanosensors, controlling elements that adjust the diameter of the vessel to maintain shear stress at the walls near some shear set point. Experiments involving both acute and chronic changes in flow through mammalian

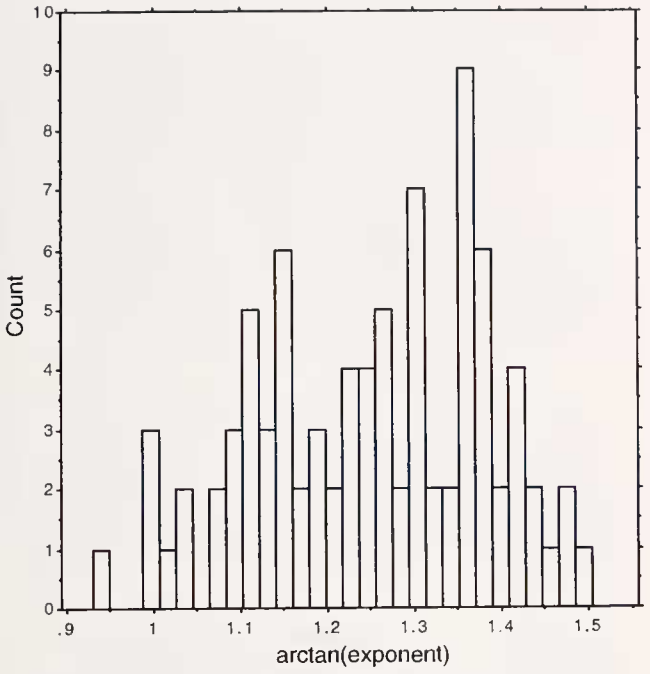

Figure 7. Frequency distribution of the arctan transformed junction exponents for 86 branch points in the arterial system of the blue crab. Note that the transformed distribution is approximately symmetrical and normal (see Table II).

blood vessels have shown that vessel diameter is altered so as to restore the shear stress to pre-experimental levels (reviewed in LaBarbera, 1993, 1994). Two distinct endothelium-mediated mechanisms (LaBarbera, 1993) apparently exist in mammals: (1) In response to *acute* changes in flow rate (= shear stress), smooth muscle tonus and thus vessel caliber is controlled by the release of prostaglandins, nitric oxide, and endothelium-derived relaxing factor (EDRF) (*e.g.,* Koller and Kaley, 1990a,b; 1991), maintaining the vasculature in a Murray's law configuration (Griffith and Edwards, 1990). (2) In response to *chronic* perturbations of flow, proliferation of both cellular and extracellular components or the breakdown of already existing elements remodel vessels, thus changing vessel caliber (*e.g.,* Kamiya and Togawa, 1980; Langille and O'Donnell, 1986; Zarins *et al.,* 1987).

Other mechanisms have been proposed to underlie endothelial responses to changes in flow rate, including changes in local ATP concentrations via flow-induced changes in diffusion boundary thickness (Mo *et al.,* 1991; Nollert and McIntre, 1992), or shear-stress-induced changes in configuration of extracellular membrane-bound proteins (Bevan and Siegal, 1991). Like the mechanosensor hypothesis, both of these hypotheses posit a response of endothelial cells to a signal dependent on the local velocity gradient; all three could result in similar responses of the system to changes in flow rate. Streaming potentials, voltages generated by the interaction of fixed surface charges with the ions carried in the fluid, are also correlated with local velocity gradients (Eriksson, 1974) and have been suggested to underlie responses of some cells to flow (Reich *et al.,* 1990; Berthiaume and Frangos, 1993).

In crustacean circulatory systems, the cells lining the vessels are separated from the vascular fluid by a continuous extracellular intima (Maynard, 1960; Johnson, 1980; Ruppert and Carle, 1983; Factor and Naar, 1990; McMahon and Burnett, 1990). Given the lack of direct contact between living cells and the fluid in the blue crab vasculature, a feedback mechanism that relies on mechanical sensing of shear stress seems unlikely. However, either changes in the local gradient of a molecule (*i.e.,* changes in diffusion boundary layer thickness) or changes in the streaming potential could be detected through the extracellular matrix lining crustacean blood vessels; both, like hydrodynamic shear stress, are dependent on the velocity gradient at the wall of the vessel and thus are attractive candidates for local signals that might control vessel remodeling in blue crabs.

The derivation of Murray's law assumes that a fully developed velocity profile is present everywhere in the system of vessels. But the parabolic velocity profile is disturbed at each branch point (LaBarbera, 1990), and the fluid must typically travel 10–80 vessel diameters downstream before complete re-establishment of Poiseuille flow (Vogel, 1981; LaBarbera, 1994). In biological systems, there are rarely 10 vessel diameters between branch points

Table III

Comparison of measured parent vessel diameters to parent vessel diameters predicted from measured daughter vessel diameters assuming a junction exponent of 3

Vessel diameter	n	*P* (sign test)	Pred/obs (mean ± SE)
All vessels	106	0.003	1.045 ± 0.013
Anatomical regions			
Anterio-ventral	35	0.18	1.056 ± 0.022
Ventral thorax, periopods	37	0.19	1.035 ± 0.027
Anterio-lateral	34	0.02	1.043 ± 0.018
Parent vessel diameter			
<167 μm	26	0.33	1.022 ± 0.022
168–268 μm	26	0.33	1.060 ± 0.035
269–430 μm	26	0.56	1.025 ± 0.027
431–785 μm	28	0.004	1.070 ± 0.020

Mean values for the ratio of the predicted diameters to the observed diameters are given for both the full data set and grouped by anatomical region and by measured size of the parent vessel. All junctions were included in the analysis. A nonparametric two-sample sign test was used to compare the ratio of observed to predicted diameters to a hypothesized value of 1.

(Zamir and Phipps, 1988; LaBarbera, 1994); hence, a static parabolic velocity profile is seldom fully re-established before the next branch point. If vessel diameter is determined by the magnitude of the local velocity gradient, downstream vessel diameters will be larger than predicted by Murray's law. In the case of the blue crab, downstream vessels are invariably daughter vessels, because the hemolymph returns to the heart through sinuses rather than veins. Given the small value of length/diameter ratio in the vessels we measured (mean 3.98), our hypothesis of the exploitation by blue crabs of the magnitude of the velocity gradient at the wall (or some variable dependent on the velocity gradient) as a signal to control vessel remodeling is consistent with the discrepancy we observed between measured parent vessel diameters and diameters predicted assuming a junction exponent of 3 (Table III).

There is no muscular tissue associated with the arterial vessels in crustaceans, with the exception of the valves at the major vascular junctions (Johnson, 1980; McMahon and Burnett, 1990; McMahon, 1992). The lack of smooth muscle in the vessel walls of crustaceans precludes an acute response similar to that observed in vertebrates. The highly elastic nature of crustacean arteries (Shadwick *et al.*, 1990) might play a role in acute control of vessel diameter to maintain a branching geometry that approximates a Murray's law system, but no experimental data are available.

The influence of fluid rheology on fluid transport system design

The branching geometry of vessels in the blue crab circulatory system is better predicted by Murray's law than is the branching geometry of mammalian vessels, where mean junction exponents vary from 1.72 to 3.09 (LaBarbera, 1994). This observation is not surprising given the rheology of the blood in these two groups. The respiratory pigment in crustaceans, hemocyanin, is transported in solution through the vascular system (Taylor, 1982), and the hemolymph contains fewer cellular components than mammalian blood. Unlike mammalian blood, where the high concentrations of erythrocytes cause the blood as a whole to act as a shear-sensitive, non-Newtonian fluid (Kiani and Hudetz, 1991), the maintenance of the respiratory pigment in simple solution in crustaceans should produce a fluid with strictly Newtonian behavior (Wells and Dales, 1976). The classic formulation of Murray's law implicitly assumes constant viscosity of the fluid (LaBarbera, 1993), an assumption violated in the smaller blood vessels of mammals. The junction exponents (mean exponents 2.47–2.93) for stromatoporoids are also closer to 3 than are those for mammals (LaBarbera, 1994); the fluid carried in the trophic fluid transport system of stromatoporoids was seawater, another strictly Newtonian fluid.

Although there have been a number of studies examining Murray's law and the maintenance of the diameter relationships between vessel branches in mammalian circulatory systems, there are virtually none for any other group of animals. More extensive work on the architecture of the fluid transport systems of animals other than mammals may yield important information on the generality of a Murray's law architecture and the variety of mechanisms involved in the production and maintenance of branching geometries that approach Murray's cost-optimized model.

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Literature Cited

Arts, T., R. T. I. Kruger, W. van Gerven, J. A. C. Lambregts, and R. S. Reneman. 1979. Propagation velocity and reflection of pressure waves in the canine coronary artery. *Am. J. Physiol.* **237:** H469–H474.

Ballard, J. W. O., G. J. Olsen, D. P. Faith, W. A. Odgers, D. M. Rowell, and P. W. Atkinson. 1992. Evidence from 12S ribosomal RNA sequences that onychophorans are modified arthropods. *Science* **258:** 1345–1348.

Berthiaume, F., and J. A. Frangos. 1993. Effects of flow on anchorage-dependent mammalian cells—secreted products. Pp. 139–192 in *Physical Forces and the Mammalian Cell*, J. A. Frangos, ed. Academic Press, Inc., New York.

Bevan, J. A., and G. Siegal. 1991. Blood vessel wall matrix flow sensor: evidence and speculation. *Blood Vessels* **28:** 552–556.

Eriksson, C. 1974. Streaming potentials and other water-dependent effects in mineralized tissues. *Ann. N Y. Acad. Sci.* **283:** 321–338.

Factor, J. R., and M. Naar. 1990. The digestive system of the lobster, *Homarus americanus*. II. terminal hepatic arterioles of the digestive gland. *J. Morphol.* **206:** 283–291.

Griffith, T. M., and D. H. Edwards. 1990. Basal EDRF activity helps to keep the geometrical configuration of arterial bifurcations close to the Murray optimum. *J. Theor. Biol.* **146:** 545–573.

Hutchins, G. M., M. M. Miner, and J. K. Boitnott. 1976. Vessel caliber and branch-angle of human coronary artery branch-points. *Circ. Res.* **38:** 572–576.

Johnson, P. T. 1980. *Histology of the Blue Crab, Callinectes sapidus*. Praeger Publishers, New York.

Kamiya, A., and T. Togawa. 1980. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am. J. Physiol.* **239:** H14–H21.

Kamiya, A., J. Ando, M. Shibato, and H. Masuda. 1988. Roles of fluid shear stress in physiological regulation of vascular structure and function. *Biorheology* **25:** 271–278.

Kiani, M. F., and A. G. Hudetz. 1991. A semi-empirical model of apparent blood viscosity as a function of vessel diameter and discharge hematocrit. *Biorheology* **28:** 65–73.

Koller, A., and G. Kaley. 1990. Prostaglandins mediate arteriolar dilation to increased blood flow velocity in skeletal muscle microcirculation. *Circ. Res.* **67:** 529–534.

Koller, A., and G. Kaley. 1991a. Endothelial regulation of wall shear stress and blood flow in skeletal muscle microcirculation. *Am. J. Physiol.* **260:** H862–H868.

Koller, A., and G. Kaley. 1991b. Endothelium regulates skeletal muscle microcirculation by a blood flow velocity-sensing mechanism. *Am. J. Physiol.* **258:** H916–H920.

LaBarbera, M. 1990. Principles of design of fluid transport systems in zoology. *Science* **249:** 992–1000.

LaBarbera, M. 1993. Optimality in biological fluid transport systems. Pp. 565–586 in *Fluid Dynamics in Biology,* A. Y. Cheer and C. P. van Dam, eds. American Mathematical Society, Providence, RI.

LaBarbera, M. 1994. The design of fluid transport systems: a comparative perspective. In *Flow Dependent Regulation of Vascular Function,* J. A. Bevan, G. Kaley, and G. M. Rubanyi, eds. Oxford University Press, Oxford. (In Press)

LaBarbera, M., and G. E. Boyajian. 1991. The function of astrorhizae in stromatoporoids: quantitative tests. *Paleobiology* **17:** 121–132.

LaBarbera, M., and S. Vogel. 1982. The design of fluid transport systems in organisms. *Am. Sci.* **70:** 54–70.

Lametschwandtner, A., U. Lametschwandtner, and T. Weiger. 1990. Scanning electron microscopy of vascular corrosion casts—technique and applications: updated review. *Scanning Microsc.* **4:** 889–941.

Langille, B. L., and F. O'Donnell. 1986. Reductions in arterial diameter produces by chronic decreases in blood flow are endothelium-dependent. *Science* **231:** 405–407.

Levesque, M. J., J. F. Cornhill, and R. M. Nerem. 1979. Vascular casting: a new method for the study of the arterial endothelium. *Atherosclerosis* **34:** 457–467.

Maynard, D. 1960. Circulation and heart function. Pp. 161–226 in *The Physiology of Crustacea Vol. I,* T. H. Waterman, ed. Academic Press, New York.

McMahon, B. R. 1992. Factors controlling the distribution of cardiac output in decapod crustaceans. Pp. 51–61 in *Phylogenetic Models in Functional Coupling of the CNS and the Cardiovascular System,* R. B. Hill, K. Kuwasaka, B. R. McMahon and T. Kuramoto, eds. Karger, Basel.

McMahon, B. R., and L. E. Burnett. 1990. The crustacean open circulatory system: a reexamination. *Physiol. Zool.* **63:** 35–71.

Mo, M., S. Eskin, and W. P. Shilling. 1991. Flow-induced changes in Ca^{2+} signaling of endothelial cells: effect of shear stress and ATP. *Am J. Physiol.* **260:** H1698–H1707.

Murray, C. D. 1926. The physiological principle of minimum work. I. The vascular system and the cost of blood volume. *Proc. Natl. Acad. Sci. USA* **12:** 207–214.

Nollert, M. U., and L. V. McIntre. 1992. Convective mass transfer effects on the intracellular calcium response of endothelial cells. *J. Biomech. Engr.* **114:** 321–326.

Oswald, R. L. 1977. Immobilization of decapod Crustacea for experimental procedures. *J. Mar. Biol. Assoc. U K* **57:** 715–721.

Reich, K. M., C. V. Gay, and J. A. Frangos. 1990. Fluid shear stress as a mediator of osteoblast cyclic adenosine monophosphate production. *J. Cell. Physiol.* **143:** 100–104.

Ruppert, E. E., and K. L. Carle. 1983. Morphology of metazoan circulatory systems. *Zoomorphology* **103:** 193–208.

Shadwick, R. E., C. M. Pollock, and S. A. Stricker. 1990. Structure and biomechanical properties of crustacean blood vessels. *Physiol. Zool.* **63:** 90–101.

Sherman, T. F. 1981. On connecting large vessels to small. *J. Gen. Physiol.* **78:** 431–453.

Suwa, N., T. Niwa, H. Fukasawa, and Y. Sasaki. 1963. Estimation of intravascular blood pressure gradient by mathematical analysis of arterial casts. *Tohoku J. Exp. Med.* **79:** 168–198.

Taylor, E. W. 1982. Control and co-ordination of ventilation and circulation in crustaceans: responses to hypoxia and exercise. *J. Exp. Biol.* **100:** 289–319.

Turbeville, J. M., K. G. Field, and R. A. Raff. 1992. Phylogenetic position of Phylum Nemertini, inferred from 18S rRNA sequences: molecular data as a test of morphological character homology. *Mol. Biol. Evol.* **9:** 235–249.

Turbeville, J. M., D. M. Pfeifer, K. G. Field, and R. A. Raff. 1991. The phylogenetic status of arthropods, as inferred from 18S RNA sequences. *Mol. Biol. Evol.* **8:** 669–686.

Vogel, Steven. 1981. *Life in Moving Fluids: The Physical Biology of Flow.* Princeton University Press, Princeton, NJ.

Wainwright, P. O., G. Hinkle, M. L. Sogin, and S. K. Stickel. 1993. Monophyletic origins of the Metazoa: an evolutionary link with the fungi. *Science* **260:** 340–342.

Wells, R. M. G., and R. P. Dales. 1976. Subunit organization in the respiratory proteins of the Polychaeta. *Comp. Biochem. Physiol.* **54A:** 387–394.

Wieringa, P. A., H. G. Stassen, J. D. Laird, and J. A. E. Spaan. 1988. Quantification of arteriolar density and embolization by microspheres in rat myocardium. *Am. J. Physiol.* **254:** H636–H650.

Zamir, M. 1977. Shear forces and blood vessel radii in the cardiovascular system. *J Gen. Physiol.* **69:** 449–461.

Zamir, M., and S. Phipps. 1988. Network analysis of an arterial tree. *J Biomech.* **21:** 25–34.

Zarins, C. K., M. A. Zatina, D. P. Giddens, D. N. Ku, and S. Glagov. 1987. Shear stress regulation of artery lumen diameter in experimental atherogenesis. *J. Vasc. Surg.* **5:** 413–420.